# Protocols for
# Biotin-coated Magnetic beads

1. Dissolve the biotin in DMSO to a final concentration of 50mg/mL.

2. Prepare pro-cooled MES buffer (0.1M, pH5.0). Mix DMSO, biotin solution, MES buffer to a volume ratio of 0.5 : 0.5 : 9. Shake the mixture violently until the solution is clear.

3. Add EDC to the mixture to a final concentration of 1%.

4. Pipet 1ml beads to an EP tube and place on the magnetic shelf, remove the supernatant when the beads are precipitated.

5. Add 1mL pro-cooled MES buffer to the beads and mix immediately by pipetting. Place the beads on magnetic shelf and remove the supernatant when the beads are precipitated. Repeat this step for 3 times.

6. Resuspend the beads with solution prepared in step 3. Shake the reaction system for 2 hours at 0 °C. Mix vigorously on the vortex mixer every 20 minutes to avoid the beads precipitating.

7. Remove the supernatant with magnetic shelf.

8. Prepare PBST buffer with 1M NaCl.

9. Do the same operation as step 5 but change the buffer to PBST (NaCl) prepared in Step 8.

10. Store biotin-coated beads at 4 °C.

If the biotin-coated beads don’ t work good, re-biotinylate the beads again

as the protocol upside.]

※We would thank Professor Lou Chunbo for providing the EDC molecular.

## Measure the binding efficiency of mSA with biotin-coated beads

1. Mix 1mg/mL mSA protein and 10mg/mL biotin-coated beads to the ratio of 10:1.
2. Shake the reaction system for 1h at 37 °C. Mix vigorously on the vortex mixer every 3 minutes.
3. After reaction, put the EP tube on a magnetic shelf. Extract the liquid supernatant.
4. Use Bradford method to measure the concentration of free mSA in the liquid. With the control group which doesn’ t contain beads in the reaction system, calculate the binding efficiency.

## Measure the binding efficiency of mSA with Atto 488 Biotin and Ultra Centrifugal Filters

1. Dissolve Atto 488 Biotin (SIGMA-ALDRICH®) powder in DMF to a final

concentration of 50 mM and store at -20 °C.

2. Dilute the stock solution to 0.5mM by PBS buffer.
3. Mix 1mg/mL mSA protein and 0.5mM Atto 488 Biotin solution to the ratio of 50:1.
4. Shake the reaction system for 1h at 30 °C.
5. Use Amicon® Ultra Centrifugal Filters (Merck) with a 10kDa cutoff to separate the protein and the free Atto 488 Biotin by centrifugation at 14000rpm for 10 min.
6. Collect the liquid at the bottom. Set 488 nm for the excitation light and 520nm for the emission light. Measure the fluorescence by Varioska® Flash (Thermo Scientific). Calculate the binding efficiency by the experimental group and control group.

[We didn’ t get good data with this method, because Atto 488 Biotin may have non-specific interaction with the membrane of the filters.]

## Measure the binding efficiency of mSA with Atto 488 Biotin and Affinity chromatography

1. Dilute the biotin stock solution to 0.5mM by PBS buffer.
2. Mix mSA protein and 0.5mM Atto 488 Biotin solution as the equal molar ratio.
3. Prepare the control reaction excluding protein.
4. Shake the reaction system for 1h at room temperature.
5. Add 2 ml Ni-NTA His-Bind® Resin (Novagen) to the column, wash the resin

twice with Binding Buffer (20 mM Tris-HCl, 0.5 M NaCl, 20 mM imidazole, 1mM β-mercaptoethanol, pH7.4).

6. Load the reaction mixture of step 4 and wash the column with the loading buffer, collect the flow through of experiment and control group.
7. Elute the protein with the loading buffer including 200mM imidazole. Collect the elution fraction.
8. Set 488 nm for the excitation light and 520nm for the emission light. Measure the fluorescence of flow through and the elution fraction by Varioska® Flash. Calculate the binding efficiency by the experimental group and control group.